The
AMERICAN JOURNAL
of
MEDICAL TECHNOLOGY

VOLUME 3 MARCH, 1937 NUMBER 2

LABORATORY RECOGNITION OF MONILIA*

By ANN SNOW, M.T.

Department of Bacteriology, University of Arkansas School of Medicine, Little Rock, Arkansas

In our laboratory we are quite frequently called upon to make mycological examinations of one sort or another. In this way a number of moniliae infections, among others, have presented themselves for study. With a view, therefore, of attempting to present something tangible along technical lines, it was thought that a few words on the moniliae might not be out of place, as coming from one technician to another.

There are a number of recognized conditions, arising as the result of infection with the so-called Moniliae. These infections are perhaps, most frequently found on the mucous membrane or the skin, but often suppurative lesions occur in vital organs such as the lungs or the kidneys. The increased interest in fungous infections makes it imperative for us as laboratory technicians to become familiar with the technical procedures of isolation and identification of the various fungi. The whole field is in a chaotic state; and for most of us, perhaps, it is a discouraging proposition to wade through the enormous conflict of opinion.

Moniliae may be associated with a variety of disease processes. At the same time it should be kept in mind that some organisms of this group may be found in the throat, mouth, and intestines of healthy individuals. Moreover, laboratory contamination by Moniliae

* *Prepared under the direction of Professor J. G. Wahlin.*

may occur. Therefore, it is advisable to make repeated examination of pathological material so as to make sure that the parasites can be found on a number of occasions. This will strengthen the assumption that the organisms are present in the lesion. In an examination of sputum for bronchomoniliasis, for example, contamination from mouth must be eliminated as far as possible. Early diagnoses are important as it is possible for the parasite to invade the blood stream with the production of metastatic abscesses. A correct diagnosis is important, since the treatment for moniliasis may be disastrous to the patient with tuberculosis. The fact that the practitioner sometimes confuses a membrane caused by a Monilia with a diphtheritic membrane also emphasizes the necessity for the laboratory technician to have a well-defined knowledge of this organism.

In this discussion I shall confine myself to such isolation and identification procedures as are applicable to the study of Moniliae. These procedures are generally tedious and may prove disconcerting to the physician who thinks a report should be forthcoming in a few days. Under such circumstances the best one can do is to turn in a preliminary report of a rather cagey nature, trusting that the final report will bear out the presumptive diagnosis. Needless to state this does not always happen, particularly where one is trying to determine the exact species.

Isolation

The first step toward the isolation of a Monilia in a suspected specimen of material which has been properly collected, naturally, is the microscopical examination. In a wet preparation, a search is made for mycelial threads of rather large size or free oval or round budding yeast-like forms. If stained by Grams method these oval or round forms will usually be Gram positive and show a wide variation in size, thus giving very little morphological distinction.

Upon finding yeast-like forms the material is then streaked on a series of plates containing suitable media. Warnock (1) used Tanner's lactic acid agar and Hill's near beer agar. We have used Sabouraud agar and dextrose agar with success. It is preferable to incubate for 48 hours as Monilia is often slower in growth than bacteria. Macroscopically the colonies are quite similar to some of the ordinary bacteria, appearing round, smooth and soft with or without pigmentation. When the plates are examined microscopically under the low power objective, the colonies of Monilia may be distinguished from those of bacteria by their thick granular appearance showing rather a distinct beaded edge due to the cells lying in the periphery. After the colony has been located, a smear is made and stained, after which the remainder is restreaked on plates to obtain a pure culture.

Identification

The identification of an organism as a Monilia is not an easy matter. This is especially true since the term Monilia has had a number of meanings and seems to be falling into disrepute in some quarters. Certain authorities recognize the name as a valid one from the standpoint of Medical Mycology, at least. Moreover, the term Monilia is generally restricted to the group Fungi imperfecti. Such restriction, however, is given less emphasis than the description of the genus and the attempted classification of the species. The result undoubtedly is that organisms having the superficial characteristics of Monilia have been called Monilia when actually they may not be Monilia at all and perhaps not even Fungi imperfecti. The moral to be drawn from this situation is simply this: Every organism having Moniliform characteristics should be subjected to an intensive study for the purpose of:

1. Properly placing it in the Fungi imperfecti.
2. Obtaining enough information about it to allow of its specific recognition.

Ascospore Formation

Since primary division is based mainly upon spore formation, the organism is planted on several media which favors their production. Perhaps, the most widely used medium at the present time is the plaster of Paris block which is kept moist with sterile water or dilute peptone solution, planted with a young culture; Gorodkova agar and slices of carrot or potato. Old cultures of the organism often prove excellent for this purpose. Before being certain that ascospores are not formed, every available method should be tried and cultures incubated at both room temperature and 37°C. A patient and diligent search is made over a period of months, if necessary. Upon the failure to find ascospores, the organism may be safely placed in Fungi imperfecti.

Monilial forms while failing to produce ascospores do produce mycelium. On solid media such as dextrose agar, the predominant type of growth is yeast-like. In agar cultures several weeks old, however, mycelium may often be seen dipping down into the medium. The cottony mycelium of the true molds is never encountered. The presence of mycelium when established is a diagnostic point in that it serves to rule out the asporogenous yeast-like forms such as Cryptococcus and Torula and also the Saccharomyces if not already eliminated by asporogeny.

Special attention must also be paid to the diameter of the mycelium. In Moniliae this is greater than one micron whereas in the

Actinomycetes a very narrow or bacillary mycelium is produced. This, then, is another differential characteristic.

Mycelium Production

In view of the necessity of establishing the presence of a mycelium of a definite size, methods of evoking its production deserve special attention. We have tried various methods such as, dextrose agar stab, gelatin stab of Shaw (2, 3) potato decoction, Dodge (4) and carbohydrate broths, but we find the gelatin as recommended by Shaw (3) and potato decoction to be the best. The gelatin stab would be applicable only to those forms which do not liquefy gelatin. It is possible, however, that by adding some dextrose to the gelatin the proteolytic activity could be inhibited so as to check the liquefaction. However, the formation of gas may interfere with this procedure. Ordinary 12% gelatin is inoculated and incubated at 20°C. or a little lower to prevent liquefaction, until growth is shown by the penetration of the medium by mycelial threads. The gelatin stab is then treated with 10% formalin in water, which is poured on the top of the culture to the depth of about one centimeter and allowed to act three to five days. This serves a twofold purpose, hardens the material making it possible to cut cross sections and kills the organisms making it safe to work with. The culture tube is broken, the glass removed, and the gelatin cut into thin cross sections free hand, with a safety razor blade, mounted in water and examined without a cover glass. The dextrose agar stab may be dealt with in the same way.

The potato decoction is made by the formula of Talice (4) as follows: Reduce 20 gm. of potato to pulp, suspend in 1000 c.c. water, boil for 15 minutes, filter through cotton, replace water lost by evaporation, distribute to tubes, and sterilize at 120° for 20 minutes. This produces both a rapid and satisfactory method, permitting a very clear and accurate study to be made of the morphology of the parasite. For this purpose a hanging drop examined with the oil immersion objective is satisfactory.

Morphology

The morphology may vary from simple yeast-like cells to that of branching mycelium. The width of the mycelium and length of the mycelial cells should be observed. In addition to this Monilae reproduce by budding cells from the mycelium. These blastospores (some time called conidia) should be noted as to size, shape, and position on the mycelium. They are often produced in clusters or whorls about the mycelium. The shape of the whorl and whether it is compound or simple should be noted, also if it is lateral or terminal.

As pointed out by Henrici (5) there is at present a tendency to limit the group of Monilia to the forms reproducing by budding from the mycelium. Such a limitation was first imposed on Monilia by Vuillemin. In some forms, such as Oidium, reproduction is by disarticulation of cells from the mycelium. Such cells are essentially arthrospores, arising by constriction of the mycelium rather than by budding. They are frequently seen arising in chains. In some quarters, however, there is a tendency to allow arthrospore formation among the Monilia. This has contributed to confusion. In the study of mycelium there may also be seen the large, thick-walled resting cells called chlamydospores. Their position should be noted as they may arise at the end of the mycelium or somewhere along the filament.

This somewhat detailed emphases on the morphology of the parasite is given since there is a tendency to break away from the biochemical classification of Castellani (6) in favor of a morphological grouping of the Moniliae. This is apparent in the work of Shaw (3) and Dodge (4). We may, therefore, summarize the group of so-called Monilia as consisting of asporogenous fungi, on solid media appearing generally as non-mycelial budding forms but under special conditions producing mycelium of considerable diameter and reproducing typically by means of blastospores. This is in conformity with Henrici and Dodge and follows the difinition laid down by Persoon.

Specific Classification

Fermentation reactions are at the present time being questioned due to a lack of constancy as may be frequently seen. The ability of some organisms to ferment certain sugars can be lost after continued cultivation. This loss may occur rapidly (three or four generations). It is interesting to note that sucrose may be fermented without gas production while dextrose and levulose form both acid and gas. This fact has been mentioned by Henrici (5). We found this to be true with an organism recently isolated from a case of bronchomoniliasis. However, in working out the fermenting powers of the same organism, a series of sugars varying from one-half to three per cent were tried; and it was found that the two and one-half to three per cent gave a much quicker reaction with a larger per cent of gas formation. This is in accordance with the observations of Watts (7) on Torula, Saccharomyces and his Oidium.

Morphology is at the present time being emphasized very strongly as a basis of classification. Since some workers have found fermentation reactions constant, there is still a tendency to correlate them

with morphology. Thus finally, in breaking the Moniliae up into species it is necessary to take all findings into consideration, namely, the morphology, gelatin reaction, formation of pellicle, sediment or rings in liquid media, fermentation reactions, giant colonies, etc.

Summary

In conclusion it may be said that laboratory recognition of the Moniliae is apt to be a time consuming process. Not only is it necessary to take into consideration such morphological features as spore formation, size and appearance of mycelium, size, shape and location of moniliform clusters, but also these findings deserve to be correlated with and not subordinated to the fermentation tests.

BIBLIOGRAPHY

1. Warnock, Fanny B.: Pathogenic Fungi, Am. Jour. Med. Technology, 2:98, 1936.
2. Shaw, F. W.: A Monilia from the Respiratory Tract, Jour. Lab. and Clinical Medicine, 12:968, 1927.
3. Shaw, F. W.: A Morphological Study of Genus Monilia. Zentralbl. f. Bakt. I Abt. Orig., 119:460, 1931.
4. Dodge, C. W.: Medical Mycology, St. Louis, C. V. Mosby Co., 1935.
5. Henrici, A. T.: Molds, Yeasts and Actinomycetes, John Wiley, 1930.
6. Castellani, A.: Fungi and Fungous Diseases, Chicago, A. M. A., 1927.
7. Watts, J. W.: Torula Infection, Am. Jour. Path., 8:167, 1932.

HEMOLOGIC OBSERVATIONS ON THE ANEMIAS AND LEUKEMIAS

III. BLOOD PATTERNS IN ANEMIA DUE TO HEMORRHAGE*

By E. A. SHARP, M.D. AND E. M. SCHLEICHER, A.B.

While anemia is in reality a clinical term implying blood depletion there is no synonym which more accurately describes the hemologic findings resulting from hemorrhage.

The blood patterns found in the anemic state induced by hemorrhage possess numerous features of unusual hemologic interest. Even though the blood findings necessarily vary with the circumstances under which blood depletion occurs, three patterns can be proposed. A description of the hemologic variation in the three blood pictures associated with hemorrhagic states will, therefore, be presented.

Posthemorrhagic Anemia—A severe anemia occurs frequently in the hemopoietically normal subject from sudden copious hemorrhage. The immediate hemologic findings in this type of anemia indicate abnormal reactions in both the erythropoietic and leukopoietic systems. As would be expected, erythrocytopenia, thrombocytopenia and hemoglobinemia can be readily demonstrated by the usual quantitative methods. The degree of depletion of the blood elements will depend, of course, upon the quantity of blood lost, the length of time elapsing between the hemorrhage and the time hemologic observations are made, the recuperative powers of the hemopoietic system of the subject affected and the effectiveness of any treatment that may have been used before the blood is examined.

It is not uncommon in this anemia to find the erythrocytes reduced to 3.0 million or less per cubic millimeter accompanied by a substantial reduction in platelets. The hemoglobin shows a balanced reduction comparable to the loss of red blood cells, whereby a color index of 0.9 or unity is usually maintained.

* *From the Anemia Laboratory, Out-Patient Department, Harper Hospital, Detroit, Michigan.*

If the blood study is made within several hours following severe hemorrhage the reticulocytes are usually below 1.0 per cent. When recuperative ability is not impaired, however, the reticulated red blood cells will usually increase to several per cent above normal within 24 to 48 hours after a bleeding episode. A reticulocytosis, therefore, is interpreted as a favorable prognostic sign of impending hemopoiesis.

Normoblasts and megaloblasts are rarely seen unless the patient was near exsanguination or vigorous hemopoietic stimulation had been applied before the observations were made. In any event, neoerythrocytosis, with the exception of reticulocytes, is rarely seen in recovery of normal blood suddenly reduced by hemorrhage.

The white blood picture in posthemorrhagic states follows the general hemologic deviation described above for the red blood elements. Immediately after the blood loss the leukocyte count may be found to be reduced to or below the lower limit of normal. The differential hemogram at this early period of study shows a normal equilibrium between the myeloid, lymphoid and monocytic cells. As spontaneous or induced hemopoiesis ensues, leukopoietic activity is observed. The leukocytic picture following acute hemorrhage is usually described, therefore, as characterized by leukocytosis and neutrophilia. The total leukocytes may reach 12,000 per cubic millimeter after a severe bleeding episode. This increase in white blood cells is accompanied by a regenerative left myeloid shift, consisting principally of segmented and stab forms with one or two per cent juvenils. Occasionally the shift to the left includes a myelocyte particularly if some antianemic stimulus has been used. The regenerative left shift is associated with hyperleukocytosis and the neutrophils exceed normal by a substantial per cent.

The red blood regeneration in this anemic state is usually rapid. An increase of 200,000 or more red blood cells daily has been observed. Hemoglobin synthesis does not always parallel a rapid regeneration of cellular elements, hence a slight hypochromemia will be observed during the recovery phase, particularly when the red blood cells reach a concentration of 4.0 to 4.5 million per cubic millimeter. As recovery from this type of anemia proceeds the thrombocytes gradually return to normal. There is seldom any appreciable delay in the return of thrombocytes to a normal level and the leukocytic picture described as typical of the initial phase of acute blood loss gradually is replaced by a normal pattern simultaneously with the recovery of the erythrocytes and hemoglobin.

Chronic Hemorrhagic Anemia—A typical blood pattern of chronic hemorrhagic states can be summarized only in the most

HEMOGRAM IN COLOR

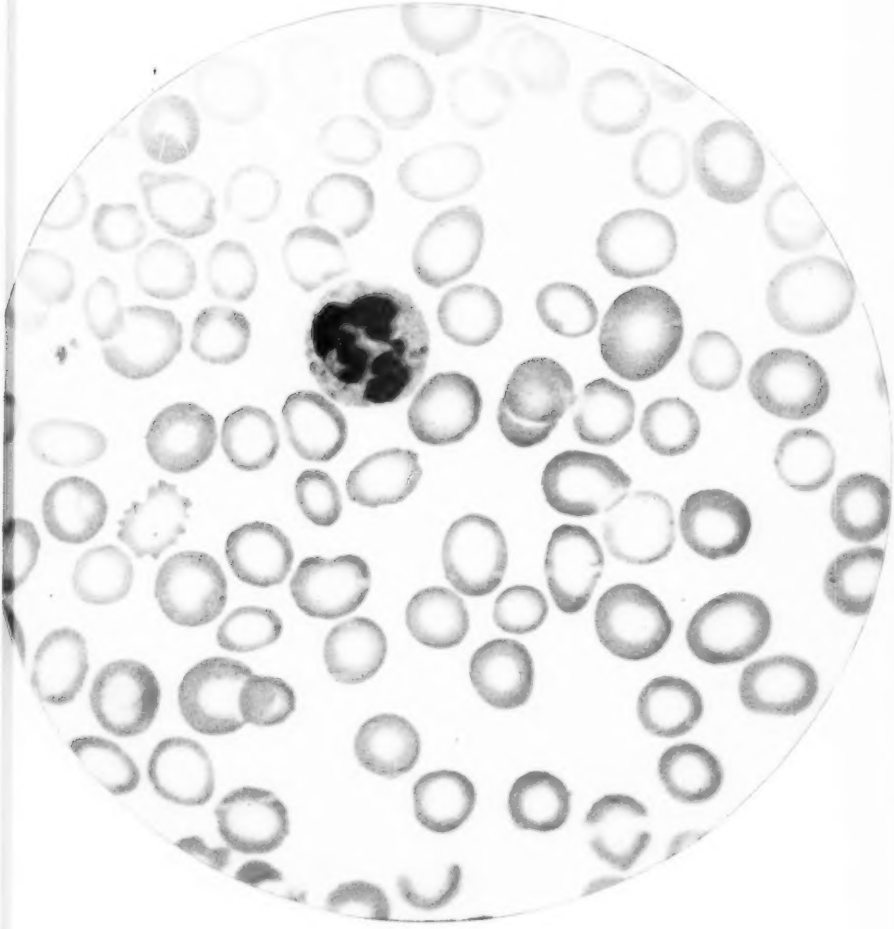
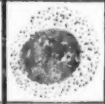
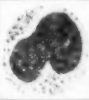


FIGURE 1

Hemogram of hemorrhagic anemia showing hemologic characteristics of transition from the acute to the chronic form. Hypochromia, anisocytosis, poikilocytosis and ovalocytosis in the red blood cell pattern and the hypermature neutrophil indicate a hemopoietic disturbance of longer duration than could be accounted for by a few bleeding episodes during the course of several days or weeks. (In accordance with the classification submitted in this essay this picture should be designated as an early phase of chronic hemorrhagic anemia.)

HEMOGRAPH

TYPE	MYEL.	JUV.	STAB	SEGM.	BAS.	EO.	LYM.	MONO.	HEMOGLOBIN %	TOTAL ERYTHROCYTES	TOTAL LEUCOCYTES
MORPHOLOGY											
NORMAL %	0	0	4	63	1	3	23	6	100	5.0	6-8000
Ca		3	16	50	0	1	28	2	85	4.0	7,600
Pa		8	25	35	2	4	25	1	66	2.8	7,400
Ro		2	12	62	1	1	20	2	68	3.8	6,400
Ra		2	12	53	1	2	26	4	82	4.2	6,700
Ho		2	18	52	1	2	22	3	88	4.0	5,700
Bo		7	15	32	3	6	32	5	51	2.1	11,250
Mi		5	20	52	1	2	18	2	40	2.0	8,700
Me		2	11	55	1	2	25	4	48	4.2	8,400
Ma		2	10	41	0	3	40	4	89	4.0	9,600
Mo		4	12	40	1	2	39	2	82	3.9	11,150
Martz		5	18	48	1	3	20	5	56	2.8	7,100
De		4	18	52	1	2	20	3	58	4.3	5,500
Bal		10	19	39	1	1	29	1	33	2.0	8,000
Bar		6	18	32	2	3	37	2	42	3.5	4,650
Zw		1	22	40	1	2	32	2	47	3.2	4,700
Se	2	9	34	38	0	0	16	1	30	2.9	10,200
We		6	14	48	1	1	27	3	42	3.8	6,400

Pretreatment blood counts on seventeen cases of hemorrhagic anemia are cited in the above hemograph. There is definite disturbance in both the red and white blood cell patterns. Imbalance is particularly noteworthy in the myeloid series with neutrophilia and left nuclear shift characterizing the majority of the counts. Patients Ca, Ra, Ho, Ma and Mo show essential hemologic findings of acute blood loss.

general terms since its protean manifestations cannot be epitomized. This profusion of hemologic deviations encountered in the blood as a result of protracted loss offers the observer, nevertheless, a most attractive field for research. In the remote past, the investigations were largely clinical but in the recent past correlations between the hemologic and clinical aspects of anemia of this variety have accumulated. Not by any means are all investigators in accord regarding the implications of altered hemopoiesis in this entity but there is full agreement about what should be regarded as its major hemologic manifestations. These will be discussed along with numerous variations.

The earliest blood pattern in chronic hemorrhagic states shows a balanced reduction of red blood cells and hemoglobin of varying degrees. The erythrocytes may remain at a level of 3.5 to 4.0 million per cubic millimeter for an indefinite time and contain a normal amount of hemoglobin. Furthermore, the size of the red blood cells is usually predominately normocytic with only an occasional macrocyte. Normablasts and reticulocytes are frequently increased. The thrombocytes show a tendency to fluctuate, frequently being found below normal concentration on one occasion but present in increased numbers at other times when serial counts are compared.

The leukogram in early stages of anemia due to continuous blood loss exhibits evidence of leukopoietic activity. The total leukocytes are found at a high normal or increased level, ranging from 8,000 to 14,000 per cubic millimeter. Associated with leukocytosis is neutrophilia embracing a regenerative left myeloid shift. The nuclear deviation of the myeloid elements may extend to the juvenil forms which may constitute from 1 to 10 per cent of the neutrophils. The monocytes are seldom present in normal numbers, but the lymphocytes are generally at or above normal percentage. The eosinophils and basophils show no significant quantitative nor qualitative variations.

The blood picture in the early phases of chronic hemorrhage, then, is essentially indicative of bone marrow stimulation. As a matter of fact, the essential hemologic difference between this early stage of chronic hemorrhagic anemia and anemia resulting from acute hemorrhage cannot be readily demonstrated except by serial blood studies. When a sufficient number of observations have been made, however, the tendency of the acute picture toward improvement and the constant retrogression in the chronic pattern serve to distinguish them.

The second hemologic phase of chronic hemorrhagic anemia is remarked by a gradual tendency of all cellular elements of the blood to show quantitative fluctuations within closer limits and a definite trend toward lower levels. Ovalocytes appear in the erythrocyte series, slight hypochromia supervenes and may progress rapidly to reach the final exaggerated phase commonly (but questionably) called achromia. A few macrocytes may be seen at this period but the predominant cells are small pale erythrocytes, both the mean diameter and the mean volume of which are reduced. Wintrobe (1) has found the mean corpuscular volume as low as 55 to 50 cubic microns in chronic hemorrhagic anemia. The progressive loss of hemoglobin during this phase causes a low color index due to a diminished corpuscular concentration. The reticulocytes are variable, a slightly increased percentage being found when counts are made about the time unusually severe bleeding episodes occur.

Very important and seldom emphasized are the changes noted in the thrombocytes as chronic hemorrhagic anemia progresses. The platelets decrease rather gradually and the fluctuations observed in the second phase seldom involve concentrations higher than normal in contrast to frequent thrombocytosis in phase one. Several qualitative deviations in the platelets can also be demonstrated. Their clumping tendency is less noticeable, the cellular stroma is less dense, giving them a hyaline appearance, and the mean diameter is found to be less than 2 microns.

The white blood cell picture shows two striking trends at this point. The total number of leukocytes decreases asd often leukopenia ensues. The neutrophils return to normal numbers or neutropenia develops with a persistent left myeloid shift. The myeloid elements show nuclear pyknosis and cytoplasmic vacuolation is not uncommon. Concomitantly with the left shift now occurs a right nuclear shift, as illustrated in hemogram 3 (Table 1). This hypersegmented neutrophil was discussed in a former essay (2). Doubtless the nuclear change is the result of karyorrhexis.

The qualitative changes in the myeloid series, coincident with a left nuclear shift including the juvenil form, necessitate at this point a modification in the designation of the "left shift" which should be referred to at this stage as "regenerative-degenerative," in contrast to its "regenerative" character in phase one.

Lymphocytes persist in a normal or increased number in the second phase of chronic hemorrhagic anemia. Their appearance does not change materially except that smaller forms are more frequent.

The eosinophils and basophils are found in normal percentages and the monocytes remain at about the same level as in phase one.

The third hemologic stage of chronic hemorrhagic states is probably better known than the patterns described above. The hemologic picture is essentially that characteristic of anhemopoiesis. The erythrocytic configuration resembles in some features the relapsed phase of Addison's anemia. Many bizarre forms of erythrocytes appear, since erythropoiesis has practically ceased. Achromia, anisocytosis, poikilocytosis, microcytosis and polychromatophilia epitomize the red blood picture.

The reticulocytes will vary quantitatively. In the event true bone marrow hypoplasia exists a diminished percentage of reticulocytes can be demonstrated in serial counts. Qualitatively, the majority show sparse reticulum with only an occasional wreath form. When anhemopoiesis is largely due to temporary exhaustion and not permanent bone marrow damage periodic reticulocytosis can be demonstrated, often as great as 10 per cent. On the other hand, in the severest depletions when bleeding has continued periodically for months or several years the integrity of the bone marrow is so impaired that practically no hemologic evidence of erythropoiesis can be demonstrated. Briefly stated, this phase of anhemopoiesis is indicative of complete bone marrow exhaustion.

The leukocytic pattern in the severe stages of chronic hemorrhagic anemia resembles in most essentials that described for the second phase. While leukopenia, neutropenia and a regenerative-degenerative myeloid shift to the left are the rule, exceptionally is found a persistency of the pattern described for the initial phase of this type of anemia.

Chronic Hemorrhagic Anemia with Complications—Hemologic observations on chronic hemorrhagic states demonstrate that the blood patterns described above are subject to variation through the mediation of complicating factors. Periodic or continuous blood loss in small amounts more often than not induces the pattern of progressive anemia which exhibits the hemologic characteristics given in detail in the previous section. The variability in blood forming systems, the duration of the anemic state and the difference in volume of hemorrhage alter materially the hemologic findings, hence the confusion patterns must be correlated.

Cessation of hemorrhage for several days will exert a definite influence on the hemologic pattern of phases one and two of the chronic type of the hemorrhagic entity. Spontaneous hemopoiesis often ensues when this occurs in which event the reticulocyte per-

centage may be as high as 10 to 12 per cent. The leukocytosis and neutrophilia frequently are replaced by normal levels, although it is rare in this variation to encounter a normal neutrophil distribution—the left myeloid shift persists scarcely without exception.

Another very important deviation from the usual hemologic configurations in chronic anemia states is observed in phases two and three when an unusually severe hemorrhage is superimposed on the current blood loss. Reticulocytosis occasionally attains a high percentage, 24 per cent has been observed, when severe hemorrhage occurs in phase two. On the contrary, copious hemorrhage in the stage of exhaustion merely accentuates the anemia in every respect. There will be seen in this contingency, however, an extensive neocytic left myeloid shift; frequently a majority of the neutrophils will consist of stabs, juvenils and myelocytes. Neoerythrocytosis, also, is seen, normoblasts and megaloblasts appear together frequently under these circumstances. This is prognostic of complete bone marrow exhaustion and is seen constantly in terminal hemorrhagic states.

Normal menstruation introduces variables in the hemologic picture of hemorrhagic anemias. When blood studies are made during the period of flow false quantitative estimates of cellular concentrations result; hence, hemorrhagic anemia should be studied intermenstrually. If counts are made during the menstrual cycle the fact should be noted, since wide variations in the quantitative determinations many times can be accounted for in this manner.

Other bizarre phenomena in the blood picture associated with chronic blood loss appear when acute and chronic infection are present; endocrinopathies cause variations, also, as do toxemias. Nutritional deficiency is also frequently a cause of early hypochromia in hemorrhagic anemia in which case the blood will show a rapid transition from the first phase to the third stage.

Summary

1. Acute blood depletion due to copious hemorrhage produces initially a uniform diminution in all cellular elements of the blood.

2. Reticulocytosis, leukocytosis with neutrophilia and progressive blood regeneration may follow acute blood loss spontaneously or result from therapy.

3. The picture of anemia produced by chronic blood loss is dependent upon the amount of blood lost, the duration of the hemorrhage and the recuperative ability of the blood forming organs.

4. Chronic hemorrhagic anemia induces bone marrow hypoplasia. Blood patterns resembling hypoplasia of the bone marrow may be encountered, also, in chronic hemorrhagic anemia immediately following a severe bleeding episode superimposed on a chronic anemia state.

5. The hemologic pattern in chronic hemorrhagic anemia shows many variations as a result of complicating factors.

6. In chronic untreated hemorrhagic conditions the blood may resemble in many respects Addisonian pernicious anemia.

BIBLIOGRAPHY

1. Wintrobe, M. M.: Erythrocyte in Man, Medicine, 9:195, 1930.

2. Sharp, E. A. and Schleicher, E. M.: Hemologic Observations on the Anemias and Leukemias. I. Myeloid Reaction in Pernicious Anemia, American Journal of Medical Technology, 2:207, 1936.

THE FOLIN BERGLUND METHOD FOR THE QUANTITATIVE DETERMINATION OF GLUCOSE IN URINE

By BERNICE ELLIOTT, B.S., M.T.
From the Bishop Clarkson Memorial Hospital, Omaha, Nebr.
F. L. Dunn, M.D., Pathologist

From the returns of the A. S. C. L. T. questionnaire (1) of 1935, 78% reported using the Benedict method for the determination of quantitative sugar in urine. Only 5% reported using the Folin Berglund method. Other methods which may be used are those of Benedict and Osterberg (2) and of Sumner (3, 4) which are colorimetric, and that of Shaffer-Hartman (3) which is based on titration.

The Benedict Osterberg method is based on the red to brown color obtained by heating the glucose solution with picric acid and alkali. The Sumner method uses a dinitrosalicylic acid reagent which is reduced by the sugar when it is heated with the urine. In the Shaffer-Hartman method, the sugar solution is boiled with a Fehlings alkaline copper solution. The residual cupric salt may be converted into cuprous iodide or be oxidized in the presence of a known amount of iodine. The iodine liberated or the excess iodine formed, if the salt is oxidized, is titrated with sodium thiosulphate.

The methods of Folin Berglund and of Sumner give approximately the same values for the sugar in normal urine, while the Benedict Osterberg method gives higher and probably less reliable values (5). There are several factors which may be responsible for the differences in analytic values. Glucose equivalents for the urinary sugar may not be the same for several analytic methods. There may be present in the urine substances which do not give colors, but which increase or decrease the amount of color given by the sugar.

In our laboratory at the Clarkson Hospital in Omaha, Nebr., we have been using the Folin Berglund method for several years on all diabetic patients. This determination has been made daily on each twenty-four hour specimen throughout the hospitalization of the patient. Since we have been pleased with the results of the test, we believe that it has great value for routine practice. The questionnaire (1) reported that 84% of those answering used the Folin Wu blood sugar method (6, 7). Since the Folin Berglund urine sugar method uses the same reagents as the Folin Wu blood sugar method, both determinations may be made at the same time.

The qualitative test is first performed on the twenty-four hour specimen to give a rough determination of the amount of sugar present. This can be done using any of the qualitative tests commonly employed. If the qualitative test is negative, we proceed to make our filtrate of the urine (7). If the urine is concentrated, 5 c.c. of the urine, 5 c.c. of N/10 oxalic acid, and 10 c.c. of water are mixed. Then 1½ grams of Lloyd's reagent are added. The mixture is shaken for about 2 minutes, and filtered through a fine filter paper, such as Whatman No. 2. If the twenty-four hour volume is large, 10 c.c. of urine, and 5 c.c. of water, or 15 c.c. of urine are used. Five c.c. of N/10 oxalic acid are added, and the treatment with Lloyd's reagent is the same as with concentrated urines. If the qualitative test shows sugar, the urine must be diluted more than when the qualitative test is negative. The addition of the N/10 oxalic acid and the treatment with the Lloyd's reagent is unnecessary if the dilution made of the urine is great. A dilution of about 1-20 is necessary if a trace of sugar or a 1+ sugar, representing from 0.15% to 0.3%, is obtained with the qualitative test. A dilution of 1-50 or 1-100 will be sufficient with a 2+ or a 3+ sugar, which represents from 0.3% to 1.0% sugar. If the qualitative test is very strong, a dilution of 1-200 or 1-400 may be necessary.

The treatment with the Lloyd's reagent is necessary to remove coloring matter, creatin, creatinine, uric acid, aldehydes, and other nitrogenous materials which might have considerable reducing power (8). This reagent is unlike most effective charcoals, in that it does not take away the sugar. It is not necessary that every trace of creatinine be removed, for relatively large amounts have absolutely no effect on the sugar method of Folin Wu. This was shown in the testing of blood (6). The shaking with Lloyd's reagent should not be continued longer than two minutes, because the reagent is gradually dissolved by the acid, and because shaking longer does not take away any more of the interfering substances. The dissolved aluminate from the reagent does not disturb the determination at any stage.

Care must be taken to obtain a dilution which will have nearly the same sugar concentration as the sugar standards. If the sugar concentration is too high, the copper content of the 2 c.c. of alkaline copper tartrate solution will be insufficient to react with all of the sugar present. If the unknown is much stronger or much weaker than the standards with which it is compared, readings made with the colorimeter do not bear a true ratio to the strengths of the solutions. If the blue color obtained after the molybdate phosphate reagent has been added is much deeper or much lighter than the

standards, the determination must be repeated, with a different dilution of the urine.

The standards used are the same as for the blood sugar. Two c.c. of each standard and of each urine filtrate or diluted urine are transferred to Folin Wu sugar tubes. Two c.c. of alkaline copper tartrate solution are added to each tube. The tubes are heated in a water bath for 8 minutes. After the tubes are cooled, 2 c.c. of the molybdate phosphate reagent are added to each tube. The cuprous oxide in the tubes dissolves immediately. As soon as the visible evolution of CO_2 has nearly ceased, usually in about one minute, dilute and compare in a colorimeter. The colorimetric formula is applied, and the amount of sugar is at once determined. We report our results in grams per twenty-four hours.

The glucose used for the standards should be the highest quality obtainable (7). The most reliable glucose for such standards is that obtainable from the Bureau of Standards, Washington, D. C. However Merck's highest purity dextrose is satisfactory. In our laboratory we use glucose from the Eastman Kodak Co. and check its use against glucose obtained from the Bureau of Standards. The sugar solution should be prepared in a 0.25% benzoic acid solution to prevent the decomposition of the sugar by molds.

The tubes used for the reduction should have a bulb large enough so that when 4 c.c. are added, the level of the fluid reaches the narrow part of the tube, and small enough so that 4 c.c. does not fill the tube beyond the constriction. Tubes which do not fulfill these requirements must be discarded. This specification is necessary to prevent atmospheric reoxidization of the cuprous oxide formed by the reducing action of the sugar (10).

An advantage of this method over the commonly used Benedict quantitative test is that the amount of sugar may be determined in normal urine. Especially when the urine is very dilute, a diabetic patient may lose considerable sugar in the urine, and still have a negative qualitative test. We are able to have a complete record of the urinary sugar output daily. A rise in the urine sugar may be noted several days before sugar is demonstrated by the qualitative test. A fall to normal sugar output may be followed daily even after the qualitative test has ceased to be positive. A colorimetric determination eliminates possibilities of error which might be introduced by overtitration or undertitration when using the Benedict method. Errors due to reoxidization, which may occur when an open dish is used for the Benedict titration, are eliminated.

Time, space, and money are saved because of the similarity of the urine and blood tests. With single determinations, however, the

Folin Berglund test requires more time than does the Benedict quantitative test.

This method can be used to advantage to determine the sugar lost during a glucose tolerance test.

Normals and well controlled diabetics usually show between 0.35 gm. and 0.75 gm. per day. A daily check on the prompt and accurate delivery of the urine to the laboratory may be made. Glycolysis may occur if the urine is not preserved with chemicals or by refrigeration. Careless preservation or loss of specimens may often be discovered if the twenty-four hour urine sugar output is less than 0.35 gm.

Summary

1. Three methods for the determination of glucose in normal urine are briefly described.

2. The procedure for the Folin Berglund method for the determination of glucose in normal urine is given.

3. Advantages of the Folin Berglund method over the Benedict quantitative sugar method are reported.

BIBLIOGRAPHY

1. Wilson, Sister M. Joan of Arc, and Brambel, C.: The A. S. C. L. T. Questionnaire. The American Journal of Medical Technology, 2:65, 1936.
2. Benedict, S. R., and Osterberg, E.: Method for the Determination of Sugar in Normal Urine, Journal of Biological Chemistry, 48:51, 1921.
3. Hawk, P. B., and Bergein, O.: Practical Physiological Chemistry, New York, P. Blakeston Co., 1931, p. 849.
4. Sumner, J. B.: More Specific Reagent for Determination of Sugar in Urine, Journal of Biological Chemistry, 65:393, 1925.
5. Everett, M. R.: and Hart, Mrs. M. O.: Determination of Sugar in Normal Urine, Journal of Laboratory and Clinical Medicine, 12:579, 1927.
6. Folin, O. K.: Determination of Sugar in Blood and in Normal Urine, Journal of Biological Chemistry, 41:367, 1920.
7. Folin, O. K.: Laboratory Manual of Biological Chemistry, New York, Appleton Co., 1934, p. 303.
8. Folin, O. K., and Berglund: A Colorimetric Method for Determination of Sugar in Normal Urine, Journal of Biological Chemistry, 51:209, 1922.
9. Folin, O. K.: System of Blood Analysis, Journal of Biological Chemistry, 38:106, 1919.
10. Hawk, P. B., and Bergein, O.: Practical Chemistry, New York, P. Blakeston Co., 1926, p. 381.

THE HERITAGE OF THE CLINICAL LABORATORY

By VIVIAN HERRICK, M.T.,

University, Alabama

When our Society adopted the creed proposed by Dr. Frederick Lamb, I became especially interested in the point "An appreciation of the heritage of the Clinical Laboratory." It seems to me that the heritage of all medicine belongs to the Clinical Laboratory and that the Laboratory as a separate entity appeared so gradually that its origin is lost in obscurity. Let us then first consider the history of Medicine in general.

The oldest historic phase of Medicine known to us is that of ancient Egypt (3400-2440 B. C.). Our sources of Egyptian Medicine are the Medical papyri. The Smith Papyrus (1600 B. C.) covers 48 cases with complete records on each case. They deal mostly with surgical conditions. The Ebers Papyrus (1550 B. C.) includes sections on eye, ear and a description of the three different stages of hookworm infection (Joachim). In addition to hookworm, Filaria, Taenia, Ascaris, other parasites are mentioned. This is the first indication of a technically-minded person or persons who were interested in differentiating the species. Let us then say that this is the first known technician or parasitologist although who or what else he may have been is not known.

The next period in medical history is called, by Garrison, The Classic Period (460-136 B. C.). This is the first record of European medicine which has its beginning in the age of Pericles and its scientific advancement centers in the figure of Hippocrates who gave to Greek Medicine its scientific spirit and its ethical ideals with which we are all familiar.

The Mohammedan and Jewish period (732-1096 A. D.). These peoples, because of religious and social ostracism, took up the study of medicine and made great advances in surgery and anatomy. As early as 707 A. D. there was a hospital at Damascus. A hospital in Cairo, The Hall of Wisdom, offered medical instruction. Here chemistry was taught and highly esteemed. Arabian medicine was the parent of alchemy, under which we may include Jabu or Gerber

(702-765 A. D.), who discovered nitric acid, aqua regia and described distillation, filtration, sublimation, water baths and other essentials of chemical procedure.

The Medieval period covers the Dark Ages, 1096-1438. This period of feudalism and ecclesiasticism is commonly decried for its servile obeisance to authority with its attendant evils of bigotry, pedantry and cruelty. The chief glory of Medieval medicine was undoubtedly in the organization of hospitals and nursing which had its origin in the teachings of Christ. The great hospital movement was inaugurated in 1198 by Pope Innocent III. Perhaps the best available sidelights upon earlier medieval medicine are afforded in the miniature paintings which illuminated their manuscripts. Among these, one from the Turin Codex, shows a master inspecting the urine in a glass while a humble-looking patient stands beside him with the urine basket in his hand. The contrast between the professional gravity of the doctor's face and the pathetic solemnity of his mute, enduring patient is one of the cleverest things in medieval art. Uroscopy or water casting was a favorite theme of the painter and wood engraver down to the 18th century. The urinal became the emblem of medical practice in the Middle Ages and urinalysis definitely a fad. As may be imagined off-hand, diagnoses were a favorite imposture of the strolling quacks who reaped a fortune from the deception. In 1302, the first judicial postmortem was performed.

Renaissance and Reformation: Medical practice during this period was rife with quackery and superstition. The first mention of a laboratory and called such, was by Libavius (1546-1616), a physician and teacher of Coburg whose Alcheymia was the first systematic treatise on the science of Chemistry. "He had," says Bolton, "a sumptuous laboratory, provided not only with every requisite for chemical experimentation but also with means of entertaining guests including such luxuries as baths, enclosed corridors for exercising in inclement weather and a well-stocked wine cellar." While we might well imagine that such luxurious surroundings would not be conducive to hard work, such is not the case. During his life he discovered stannic chloride, analyzed mineral waters, wrote a city pharmacopoeia and was one of the first to suggest blood transfusions. Paracelsus and some of his followers owned private laboratories in which they concocted secret remedies and did experiments also using them for business places where they sometimes operated as pawnbrokers, usurers, sold calendars and cast horoscopes. Belonging to this time are Leonardo Da Vinci and Vesalius whose works in anatomy became the starting point for modern medi-

cine. Laws were made to govern the practice and in 1522, the College or Commonalty of the Faculty of Physics of London set up what is the earliest and most important code of ethics. Epidemics were recognized as being of contagious nature and laws of general hygiene were passed. Hospital construction reached perfection in the 15th century. In 1450, Krebs suggested timing the pulse and weighing the blood and urine. This then was the period in which medicine began its advance from empiricism to the dignity of a science.

The 17th century marks the age of individual scientific endeavor. The invention of the microscope opened a new departure for medicine in the direction of the invisible world. The early history of the microscope is somewhat complex and indefinite. In 1590, a compound microscope was invented by Hans and Zaccharias Janssen. It was greatly improved in 1621 by Conelius Drebbel. The earliest microscopist is said by Garrison to be Athanasius Kircher (1602-80), who in his "Scrutinium pestis," 1658, details seven experiments upon the nature of putrefaction. Leeuwenhoeck (1632-1723) of Delft, Holland, a linen draper and janitor, devoted his leisure to the study of natural history. He had some 247 microscopes with 419 lenses most of which were ground by himself. He once sent 26 microscopes to London as a present to the Royal Society of which he was made a member in 1680. During his long life, he sent 375 scientific papers to the Royal Society and 27 to the French Academy. These papers contain a vast amount of work on animalculae and plant history and many discoveries of great importance to medicine. He was first to give a complete description of the red cells, to see protozoa and classify bacteria according to shape. He demonstrated the capillary anastamoses between the arteries and veins which Malpighi had already seen in 1660 without attaching much importance to it. It was Malpighi's discovery and Leeuwenhoeck's thorough work on the capillary circulation which finally completed Harvey's demonstration on the circulation of the blood. Malpighi (1628-94) was the greatest of the early microscopists and his work in embryology and anatomy definitely marks him as the founder of Pathology.

Intravenous injection and blood transfusions had their scientific origin in the 17th century, the first successful one being performed from sheep to man by Denis in 1667. Boyle defined chemical elements and Willis described the sweetish taste of diabetic urine although the latter was known to the early Arabian doctors. The 17th century marks the rise of many European universities and the origin of scientific societies for research and experimentation and

the appearance of scientific periodicals. Many European physicians emigrated to America, and Harvard College (1636) and William & Mary (1693) were established. The first hospital in the new world was erected by Cortez in Mexico City in 1524 and the first hospital in what is now the United States was established on Manhattan Island in 1663.

The Eighteenth Century: The age of theories and systems. Spallanzani through his scientific experiments defeated the doctrine of spontaneous generation. Great epidemics of small pox, diphtheria, typhus and dysentery swept over Europe, Asia and America. In 1706, the first laboratory of Marine Zoology was established at Marseilles. Hewson, the physiologist, made his experimental inquiries into the properties of blood. In 1770, Cotugno demonstrated the presence of albumen in urine. In 1773, Lind showed the possibility of the prevention of typhus. In 1796, Jenner vaccinated William Phipps and founded preventive medicine. During this century quarantines were inaugurated and isolation hospitals established and the first municipal boards of health appeared. The conquest of disease was definitely under way in the eighteenth century.

The nineteenth century marks the beginning of the organized advancement of science. In 1818, De Riemer introduced frozen sections. In 1819, Lobstein was called to the first chair of Pathology at Strassburg and later the same year a chair was established at Vienna to which Biermayer was appointed. In 1823, the Chevallier brothers invented the achromatic microscope. Gerhard differentiated typhus from typhoid in 1837 and in 1839 the Royal Microscopical Society of London was founded. Discovery followed hard on the heels of other discoveries so that only some of the more outstanding, which are related to the history of laboratory medicine, will be chronicled here. In 1843, Oliver W. Holmes pointed out the contagiousness of puerperal fever. In 1845, Virchow and Bennett described leukemia, Buchanan investigated the coagulation of the blood and Lagenbeck detected actinomyces. In 1846, the Pathological Society of London and the American Medical Association were organized. In 1847, Semmelweis discovered the pathogenesis of puerperal fever and the Archives of Pathology was founded in Berlin by Virchow. In 1848, Fehling introduced his test for sugar in the urine. In 1849, Roger and Davaine discovered the anthrax bacillus. Between 1850 to 1860, Funke discovered hemoglobin; Babo demonstrated the rapid separation of blood corpuscles from serum by centrifugation; Cohn established the vegetable nature of bacteria; the Crimean war took place and gave us Florence Nightingale with whose history and works we are all familiar; Burmeister classified insects; the Bunsen burner was invented; typhoid fever infections were traced to milk and Petters discovered acetone

in urine. Pasteur in 1860-61 demonstrated the presence of bacteria in air and described anaerobes. During this decade many medical schools, hospitals, medical museums and societies were formed over the United States and the whole world. During the period of the Civil War in the United States, no notable discoveries were made. In 1871, Weigert introduced the successful staining of bacteria with carmine. This was followed in 1872 by Merck's work with the methyl violet. Obermeier in 1873 announced his finding of the causative organism of relapsing fever. In 1874, Ehrlich introduced dried blood smears and an improved technique for methyl staining. Almost simultaneously with Losch's discovery of parasitic amoeba in 1875, the first public health laboratory was established by Corfield in England. Shortly after this, Koch obtained his first pure cultures of anthrax bacilli on artificial media and the following year, Pasteur announced the finding of the bacillus of malignant edema. Bacteriology was introduced to the United States in 1878 by Welch, Prudden, Sternberg and Salmon. In 1879, Neisser discovered the gonococcus and together with Hansen the bacillus Leprae. During the last two decades of the 18th century, Pasteur described the streptococcus, staphylococcus and pneumococcus, producing a vaccine against anthrax and his now famous vaccine for rabies; Laveran discovered the parasites of malaria; Eberth the bacillus typhosus; Koch formulated his inestimably valuable postulates, discovered the tubercle bacillus and introduced the solid plate culture and steam sterilization. Metschnikoff advanced his theory of phagocytosis, Klebs discovered the diphtheria bacillus and Kjeldahl devised a method for the estimation of nitrogen. Bizzoero noted the blood platelets, Loeffler and Schutz—the B. Glanders, Ewald and Boaz perfected their test meal and the examination of the gastric contents. Thus we see that the 19th century merged into the present and the discoveries at the turning of the century were so intimately connected with and dependent upon those of the last that a distinct chronological account is difficult.

The twentieth century ushers in the beginning of organized preventive medicine. The most notable things about recent medicine have been the trend toward co-operation and the fact that nearly every improvement which has been made is of a prophylactic spirit. This is the era of experimental medicine, embryology, cytology and tissue cultivation. The leading spirit of recent pathology is conceded to be Aschoff of Berlin. Folin and Wu since 1919 have been prominently identified with the field of blood chemistry having developed the use of the colorimeter. Parasitology has been brilliantly represented in recent years by Ronald Ross and his work with Malaria and mosquitoes; Bruce with the tsetse fly and nagana;

Walter Reed and his associates and Hideyo Noguchi with yellow fever and the mosquito. The study of hematology was founded by Georges Hayem of the Paris faculty, the subject being practically unknown when he took it up in 1875. The story of the Conquest of Syphilis is one of the most interesting, and it workers some of the most outstanding men of Science in the history of Medicine. This scourge which had struck mercilessly the whole world for several centuries was finally conquered by men who preceded us, by only a few years. Fritz Schaudinn in 1905 crowned his life work by the discovery of the spirocheta pallida of syphilis. His discovery of this almost invisible parasite was due to his incomparable skill in technique and staining method and his infinite capacity for taking pains. The causal relation of this organism was rapidly established by thousands of confirmatory observations made by enthusiastic microscopists the world over. The second important worker in the creation of serology was Paul Ehrlich. He was an indifferent student whose main interest lay in dyes and tissue. He greatly improved staining methods of the time and gave blood work added impetus by the use of fixed blood smears and his vital staining technique. He was the first to discover the acid fast property of the tubercle bacillus. His greatest work was his side-chain theory which proved of such immense value in developing the science of immunity and serum reaction. August von Wassermann did not hesitate to affirm that without it he could never have devised his reliable diagnosis of syphilis, that which is familiar to all of us, The Wassermann Reaction. The crowning glory of this whole conquest of syphilis must of course end on the happy note of the discovery of Salvarsan by Ehrlich. We cannot fail in connection with serology to mention the name of Bordet who was a great pioneer having discovered bacteriolysis, hemolysis and, with Gengou, the fixation of complement. Bacteriology is now a recognized, highly organized science of immense practical efficiency. The advances and findings in technique and theory present a staggering and awe-inspiring array.

Webster tells us that a heritage is an estate that passes by descent, that which is inherited, any birthright, an inherited quality, condition or lot. What then is this lot of ours, this heritage which we are to appreciate? It is a profession that is a science. Intimately connected with the history of medicine is the illustrious roll of its martyrs to communicable diseases: Servetus and Semmelweis who died for their opinions; Carrion (verrugas), Lazear and Noguchi (yellow fever); Yersin and Mueller (bubonic plaque); Carbne (malta fever); McFayden (typhoid and malta); McClintick (Rocky-mountain fever). All of whom lost their lives investi-

gating the diseases with which their names are associated. Leeuwenhoeck worked for twenty years without an audience and he wrote to the Royal Society on being elected a fellow, "I will serve you faithfully during the rest of my life." He was completely honest, startingly accurate and blessed with a common sense. Spallanzani said, "If I set out to prove something, I am no real scientist. I must learn to follow where the real facts lead me; I have to learn to whip my prejudices." And he followed them so accurately and well that he lived to disprove the theory of spontaneous generation. Roux performed his heart-breaking experiments with diphtheria serum with the courage of a Richard. Lister, who applied the antiseptic principles to surgery, at Pasteur's jubilee in 1892 paid a feeling tribute to the man whose work he had been first to appreciate. The character of Lister was one of rare nobility and he too belongs in the great hippocratic tradition. Ours then is the heritage of these men of broad vision, who visualized the whole human race as their patients for wherever the art of medicine is loved, there also is love of humanity. A heritage of rugged work, hard controversy, self-possession, unselfish and tolerant nature, a wholesome and scientific spirit, high and varied achievement embedded in the solid rock of truth, an ability to endure opposition with serene fortitude and greatest of all, a high ideal of service. In the words of Pasteur, whose character and life belongs intimately to us by inheritance, "The future belongs to those who shall have done most for suffering humanity." All of the great tradition of medicine and especially that of the men who are responsible for our profession, belongs to us, not by an accident of birth but because we have chosen it as our own. May we, in our turn, be able to add to that magnificent roll and bequeath to those who will follow something worthy of our predecessors.

SOURCES

F. H. Garrison—History of Medicine.
V. Robinson—The Story of Medicine.
P. De Kruif—The Microbe Hunters.

EDITORIALS

"WHAT CAN I WISH TO THE YOUTH OF MY COUNTRY WHO DEVOTE THEMSELVES TO SCIENCE?"—PAVLOV

In "The Bequest of Pavlov to the Academic Youth," written only a short time before his death, at the age of 87 years, he gave the substance of a life filled with reasoning, understanding and learning. We laboratory workers would do well to weave into our beings these simple principles, *viz.*, Pavlov's Postulates: Gradualness, Modesty and Passion.

Firstly, GRADUALNESS (Patience): Never expect to be a successful scientific worker unless an adequate foundation has been laid. Position gained by "bluff" will inevitably fail. Do not become just an accumulator of facts. By real thought analyze their occurrence and their laws; using the knowledge gained for building.

Secondly, MODESTY: However great the praise, remember, the great amount of scientific data acquired through all the years, is infinitely small compared to the vast amount yet to be acquired and though it seems that we are wise we really are blissfully ignorant. Never disregard friendly advice or help.

Thirdly, PASSION: Science demands all that man can give. Work with a passion, and remember, that if one had more than a single life to give to science they should all be given with alacrity.

REFERENCES TO AID IN THE WRITING OF ARTICLES

Frequently medical writers find it difficult to obtain references in past medical literature which will help them in preparing a paper.

They would like such references if they could get them.

The largest medical library in the United States is in the Surgeon General's office. The second largest, we believe, is in the New York Academy of Medicine. The services of the Academy library is available at all times. The American Medical Editors' and Authors' Association, through its Director, Dr. Harold Hays, 133 East 58th Street, New York City, can arrange to supply references or abstracts on any medical subject. Please write to Dr. Hays for information if you are interested. The cost will depend upon how much information is desired. The original bill rendered by the Academy will be sent to the individual making inquiry. The Association makes no charge.

BOOK REVIEWS

SYNOPSIS OF CLINICAL LABORATORY METHODS. By W. E. Bray, B.A., M.D., Professor of Clinical Pathology, University of Virginia; Director of Clinical Laboratories, University of Virginia Hospital. Published by the C. V. Mosby Co., St. Louis. Pp. 324. Fabricoid binding. Price $3.75.

This 1936 publication is a small, concise volume containing all the usual laboratory technique in addition to the more recent laboratory methods such as the modified Friedman test for pregnancy, pneumococcus typing, sedimentation velocity, measurements of erythrocytes in anaemia, the Schilling and Arneth hemograms and others. There are thirty-two text illustrations and eleven color plates. One chapter is devoted to tests for various metallic poisons and foreign substances, with the basophilic aggregation test for lead poisoning given in the chapter on Hematology. There is one chapter on Surgical Pathology and another on Indicators, Stains and Staining Solutions, Reagents, Removal of Laboratory Stains, Atomic Weights, Table of Equivalents and a Table of Normals. The book would be found useful in any clinical laboratory as a ready reference manual. It is strictly up-to-date and written in a concise "laboratory" style.

A TEXTBOOK OF BACTERIOLOGY, WITH A SECTION ON PATHOGENIC PROTOZOA. By Hans Zinsser, M.D., Professor of Bacteriology and Immunology, Harvard University Medical School; Consulting Bacteriologist to the Peter Bent Brigham Hospital and the Children's Hospital, Boston, and Stanhope Bayne-Jones, M.D., Professor of Bacteriology, Yale University Medical School, Master of Trumbull College, Yale University, New Haven, Connecticut. Publishers, D. Appleton-Century Co., New York. Pp. 1226, 174 illustrations.

This seventh edition is a balance between the purpose of the first edition which was "primarily a treatise on the fundamental laws and technique of bacteriology, as illustrated by their application to the study of pathogenic bacteria" and the sixth edition which was a development of the book into a manual of infectious diseases. The seventh edition records "the advances of the fundamental science of bacteriology and 'selects' for special discussion the relation of old

and new bacteriological knowledge to the diagnosis, specific therapy, epidemiology and prevention of infectious disease."

There are sections on the general biology of bacteria and the general methods of bacteriology, infection and immunity, pathogenic micro-organisms, rickettsia diseases, spirochetes, mycology, viruses, diagnostic methods of protozoology and technical methods of bacteriology, immunology and serology.

This book, formerly by Hiss and Zinsser and now by Zinsser and Bayne-Jones, has been a standard textbook of bacteriology for the past twenty-seven years. The seventh edition is by no means an exception to the rule. Many important advances in knowledge of immunology, bacterial variability and dissociation, correlation of changes in bacterial form and cultural characteristics with virulence and with chemical modifications in the bacterial antigen, the chemical assay of antigens and many other advances are included in the text. Students and practitioners of medicine and public health will find the book invaluable.

ABSTRACTS

THE ERYTHROCYTE SEDIMENTATION RATE IN ESTIMATING ACTIVITY IN PULMONARY TUBERCULOSIS. Winfield O. Kelley. American Rev. Tub., 34:489 (Oct.) 1936.

In the study of 290 cases of pulmonary tuberculosis, 1381 sedimentation rates were determined. A close correlation was found between the rate and study of the activity of the tuberculosis process by roentgenological examination. The leucocyte count was not as accurate as the s.r. but presence of coarse râles, symptomatic activity and sputum compared favorably. In some cases relapses were indicated by s.r. before shadows appeared in X-Ray. Pleurisy gave abnormal s.r. Only one reading was taken and that at the end of 1 hour.

NEWER KNOWLEDGE ON THE ANTIGENIC CONSTITUTION AND IMMUNITY REACTION OF BACTERIA AND THEIR CLINICAL BEARING WITH SPECIAL REFERENCE TO B. TYPHOSUS. P. 73-101 (Part Two) by S. C. Seal, M.B.—Calcutta Medical Journal, Vol. 31, No. 2, Aug., 1936.

In view of advance in knowledge of the antigenic constitution of bacteria it is suggested that modifications be made in the manner outlined by the author as regards sero-diagnosis, Widal test and preparations of sera, vaccines, etc. Variation in results by different workers is due mainly to absence of any fixed standard for these methods. It is urged that methods be standardized on a basis of recent advances.

HEMATOLOGICAL STUDIES IN INDIAN WOMEN. A preliminary report on the determination of the normal reticulocyte value in eighty-six healthy Bengali women. By Jyoti Dhar, B.Sc., M.B. Calcutta Med. Jour., Vol. 31, No. 3. Sept., 1936.

The average reticulocyte count was 0.945 per cent of red cells, the hightest being 2 per cent and the lowest 0.3 per cent. The average counts in this series are much higher than those previously reported by less reliable methods.

CHANGED PHYSICAL PROPERTIES OF PLASMA PROTEINS IN NEPHROSIS: M. C. Ehrström. Acta Medica Scandinavica, Dec. 19, 1936, p. 427.

Ehrström discusses the method and gives results in percentage of discoloration and effects of other substances in plasma.

BLOOD AND PLASMA VOLUME CHANGES IN ECLAMPSIA: W. J. Dieckmann. American J. Obstetrics and Gynecology, Dec., 1936, p. 927.

Author found that relative or absolute concentration of blood occurs in eclampsia and can be demonstrated by blood and plasma volume determinations, but is best revealed by serial determinations of hemoglobin, cell volume or serum protein concentration. Blood concentration is intimately associated with the convulsions, coma, oliguria and various cerebral, visual and gastro-intestinal symptoms. Clinical improvement follows blood dilution. Three patients died in whom dilution could not be maintained.

COMPARATIVE VALUES OF CLINICAL AND POSTMORTEM BLOOD CULTURES: C. G. Burn and D. F. Harvey. Journal of Infectious Diseases, Nov.-Dec., 1936, p. 296.

Factors causing errors and discrepancies are discussed from a study of 212 cases having clinical and postmortem blood cultures.

PRACTICAL SIGNIFICANCE OF SEDIMENTATION OF ERYTHROCYTES: H. Reichel. Wiener Klinische Wochenschrift, Dec. 11, 1936, p. 1517.

Simplicity and method of sedimentation reaction are discussed. Accelerated sedimentation observed in (1) all inflammatory processes, (2) necroses and cellular disintegration and (3) parenteral resorption of protein. Sedimentation speed is non-specific reaction, like fever and leucocytosis, and is never accelerated in healthy individuals but normal speed is not necessarily a sign of health, for the sedimentation speed is accelerated only if the protein composition of the plasma is changed.

NEW, SIMPLE METHOD FOR COUNTING OF PLATELETS: K. Leuggenhager. Schweizerische medizinische Wochenschrift, Dec. 19, 1936, p. 1289.

Sodium citrate sol. (3.8%) is used with erythrocyte counting pipet. Solution should be sterile and centrifugated before each use. Advantages: (1) rapidity, (2) direct counting in chamber with erythrocytes in same specimen, (3) great exactness of the method.

NEWS AND ANNOUNCEMENTS

REGISTRY OF MEDICAL TECHNOLOGISTS OF THE AMERICAN SOCIETY OF CLINICAL PATHOLOGISTS

The office of the Registry is now receiving applications for the April, 1937, semi-annual examination.

Three hundred and fifty-three new names were added to the roster of certified Medical Technologists as the result of the examination last October.

The long awaited MODEL CURRICULUM for training in medical technology has at last been issued after long preparation and is now available to students, instructors, and those registered Medical Technologists interested in keeping abreast of their profession. A price of $1.25 per copy has been established to cover the cost of publication and postage. The Manual has a special binding so that the leaves remain flat wherever opened. It is also supplied with ample space for notes.

The rate of growth of the Roster of the Registry warrants the safe prediction that within a few years all qualified laboratory workers in the United States and Canada will be enrolled under its banner. While the Registry does not sigh for other worlds to conquer, it is interesting to note that inquiries and in a few cases applications have come in from far off Shanghai, not to mention the Canal Zone, England, Germany, India, and of course our insular possessions.

NATIONAL

A special Executive Session called by the President, Frieda Claussen, was held in Chicago, February 13th and 14th. Those in attendance were F. Claussen, Minn.; Sr. Alcuin, Minn.; C. Seguin, Ill.; J. H. Conlin, Mich.; L. Gifford, N. J.; F. Ward, N. J.; H. Macko, Ohio; B Elliott, Neb.; J. T. Fitzgerald, Maine; and A. Snow, Ark.

A report on the revision of the Constitution and By-Laws was given by B. Elliott, Chairman of the Committee. A discussion followed, the results of which will be sent out to the members sixty days prior to the Annual Meeting.

In the absence of Robert Jenkins, Chairman of the Affiliation Committee, B. Elliott presented an article providing for State Affiliation to be added to the Constitution of the American Society of Medical Technologists.

Reports were given by C. Seguin, Treasurer and F. Ward, Chairman of the Executive Committee.

Dr. I. Davidsohn, a member of the Board of Registry, met with the group at which time problems of the Society were presented and discussed.

SISTERS' RESERVATIONS AND ENTERTAINMENT COMMITTEE

In anticipation of a large number of the sisterhood who plan to attend the FIFTH ANNUAL CONVENTION which will be held in June, the following committee has been appointed: Sister M. Alcuin, St. Mary's Hospital, Duluth, Minnesota, Chairman; Sister M. Inez, Mercy Hospital, Canton, Ohio; and Sister M. Gonzaga, St. Joseph's Mercy Hospital, Detroit, Mich. For further information, write direct to Sister M. Alcuin.

PAPERS AND EXHIBITS

If you are planning to present a paper or exhibit at the Atlantic City Meeting, June 7-9, and have not communicated with Luella Gifford, M.T., 339 Boush Street, Norfolk, Va., Chairman of the Program Committee or Paul Brown, M.T., Newark Beth Israel Hospital, Newark, N. J., Chairman of the Scientific Exhibit Committee, we urge you to do so at once. Each member is invited to take an active part.

ENTERTAINMENT COMMITTEE

The following Entertainment Committee for the Annual Convention has been appointed:

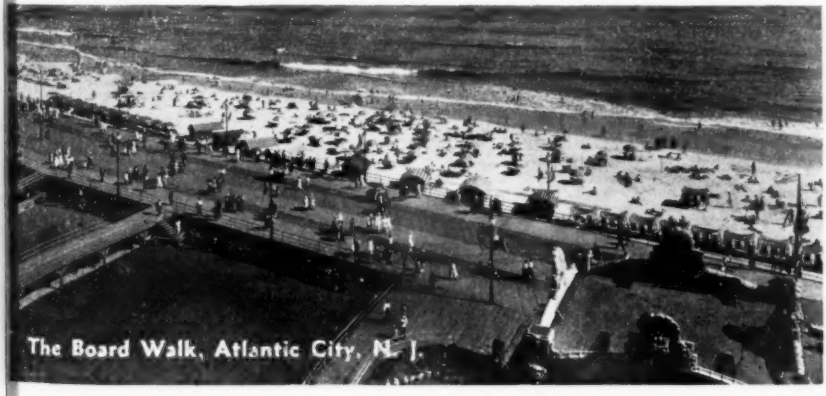
Chairman, D. Zoll, M.T., 1420 W. Girard Ave., Phila., Pa.; D. Bowman, M.T., Utica State Hospital, Utica, N. Y.; F. Stenig,

M.T., Atlantic City Hospital, Atlantic City, N. J.; M. Baker, M.T., 306 Waggoner Bldg., Wichita Falls, Texas; P. Stanley, M.T., Presbyterian Hospital, Newark, N. J.

NOTICE

The First International Conference on Fever Therapy will hold its sessions on March 29th, 30th and 31st, 1937, at the College of Physicians and Surgeons, Columbia University, New York City.

Baron Henri de Rothschild, of Paris, is General Chairman of the International Conference on Fever Therapy. Dr. Walter M. Simpson, Dayton, Ohio, is Chairman of the American Committee.



A VIEW FROM THE CONVENTION CITY OF 1937

s

g
al
ll

g
d
r.
n